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## LuxS Affects Flagellar Phase Variation Independently of Quorum Sensing in *Salmonella enterica* Serovar Typhimurium<sup>▽</sup>

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**LuxS catalyzes the synthesis of the quorum-sensing signaling molecule autoinducer 2. We show that in *Salmonella enterica* serovar Typhimurium, deletion of the *luxS* gene polarizes flagellar phase variation toward the more immunogenic phase 1 flagellin. This phenotype is complementable by *luxS* in *trans* but is independent of quorum-sensing signals.**

Quorum sensing in bacteria involves the production and detection of signaling molecules, called autoinducers (AI), which allow bacteria to coordinate gene expression (1, 2, 15, 20, 30). Following detection of a critical density of AI, bacteria coordinate their gene expression and behave in a “multicellular” fashion. Quorum sensing is implicated in the regulation of a range of processes from bioluminescence to virulence (11, 27, 32). LuxS is involved in the production of the AI-2 signal molecule (4, 21, 25) and also plays an important role in central metabolism, being part of the activated methyl cycle (29, 31). Taga et al. demonstrated that *luxS* in *Salmonella enterica* serovar Typhimurium affects the expression of, surprisingly, only a single operon encoding the Lsr AI-2 sensor system (28). *S. enterica* serovar Typhimurium is an important food-borne pathogen (18). *Salmonellae* swim using two different flagellar subunit types, either FlhC (phase 1) or FlhB (phase 2) (3, 33). Flagella have previously been implicated in invasion of host cells by *Salmonella* (14, 17). They also stimulate the host immune response by binding to Toll-like receptor 5 (23). Recently, FlhC has been shown to play a unique role in macrophage-induced bacterial killing by its unorthodox secretion through the type 3 secretion system (8, 19). In this study, we highlight the contribution of *luxS* to flagellar phase variation in a quorum-sensing-independent manner.

A complete deletion of *luxS* from the start codon to the stop codon was constructed in *S. enterica* serovar Typhimurium SL1344 (Table 1) using  $\lambda$ -red recombination (6), to generate SL1344LS. The deletion was confirmed by PCR, Western blotting with LuxS antibodies, and AI-2 detection using the well-established *Vibrio* bioluminescence reporter bioassay (24).

We assessed the secretion profiles of SL1344LS and its isogenic parent by sodium dodecyl sulfate-polyacrylamide gel

electrophoresis and Coomassie blue staining of proteins. This revealed a major difference in the levels of FlhC and FlhB flagellins, with FlhC being predominant in SL1344LS as identified via matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (Fig. 1A). No differences in motility were observed between SL1344 and SL1344LS (data not shown) (22). We assessed phase variation using triphenyltetrazolium chloride (TTC) indicator plates (10). The *fliC::mudJ* reporter was transduced from TH1077 (10) into either SL1344 or SL1344LS, giving rise to SL1344F and SL1344LSF, respectively. A single Lac<sup>+</sup> (white on TTC) or Lac<sup>−</sup> (red on TTC) colony of either SL1344F or SL1344LSF was grown to mid-log phase in LB and plated for single colonies on TTC plates. Experiments were repeated in triplicate. Although in SL1344F inversion to phase 2 predominates, in SL1344LSF we discovered a bias toward expression of phase 1 flagellin (Fig. 1B). This switching phenotype was complementable in *trans* using *luxS* expressed from its natural promoter (Table 1, pBR*luxS*), which restored switching frequencies back to parental levels, highlighting the importance of *luxS* in modulating flagellar phase variation (Fig. 1B).

To determine whether AI-2 or other diffusible signals in SL1344 supernatant supplemented with LB (reconstituted supernatant) could complement flagellar phase variation, SL1344LSF was cultured for 16 h in reconstituted supernatant to stimulate physiological adaptation mediated by AI-2 or another diffusible metabolite. Phase variation frequencies were then determined using the TTC indicator plate method and reconstituted medium to grow the relevant strains. Growing SL1344LSF in reconstituted supernatants did not reinstate parental flagellar phase variation frequencies (Fig. 1B), suggesting that neither AI-2 nor other diffusible signals mediate flagellar phase variation under these conditions.

Since flagellar phase variation in *S. enterica* serovar Typhimurium is Hin recombinase dependent, we determined *hin* transcript levels in SL1344LS by Northern dot blots (16) and found no significant differences (Fig. 2). Phase 1 and phase 2 transcript levels correlated well with the observations made independently by the methods described above (Fig. 1 and 2). This may imply the existence of either an indirect posttran-

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TABLE 1. Strains, plasmids, and primers used in the study

Strain, plasmid, or primer	Genotype, description, or sequence <sup>a</sup>	Reference	Restriction site/comment
<b>Strains</b>			
SL1344	Parent strain	13	
SL1344LS	SL1344 <i>luxS</i>	This work	
TH1077	LT2 <i>fliC5050::MudJ</i>	9	
SL1344F	SL1344 <i>fliC5050::MudJ</i>	This work	
SL1344LSF	SL1344 <i>luxS fliC5050::MudJ</i>	This work	
SL1344LSF(pBR322)	SL1344 <i>luxS fliC5050::MudJ</i> with pBR322	This work	
SL1344LSF(pBR <i>luxS</i> )	SL1344 <i>luxS fliC5050::MudJ</i> with pBR <i>luxS</i>	This work	
<b>Plasmids</b>			
pBR322	Cloning vector	26	
pBR <i>luxS</i>	pBR322 with HindIII-BamHI fragment containing <i>luxS</i> region	This work	
<b>Primers</b>			
OMW41	GCGAAGCTTACCGAGCCGTTTGCCGCGTGG		HindIII
OMW42	GCGGGATCCATTTAACAGGCCAGGCATTAC		BamHI
STM2817FOR	ATGCCATTATTAGATAGCTT		<i>luxS</i> /Northern
STM2817REV	CTAATACGACTCACTATAGGGAGATGGTCGCGCATAAAGCCAGC		<i>luxS</i> /Northern
STM2772FOR	CCTGGTGGCGTTAATATCAG		<i>hin</i> /Northern
STM2772REV	CTAATACGACTCACTATAGGGAGAGCCCTCCAGTCGTCCTTGC		<i>hin</i> /Northern
STM1959FOR	AGTACTTTTAAAGCCTCGGC		<i>fliC</i> /Northern
STM1959REV	CTAATACGACTCACTATAGGGAGAAGCGGGGAAGTCGCACCGCC		<i>fliC</i> /Northern
STM2771FOR	TCGGGTCTTGATGATGCAGC		<i>fliB</i> /Northern
STM2771REV	CTAATACGACTCACTATAGGGAGAGCCGCAAGGGTTACTGTACC		<i>fliB</i> /Northern

<sup>a</sup> Restriction sites are underlined.

scriptional effect on Hin recombinase or a Hin-independent regulatory mechanism. The importance of phase variation in *Salmonella* pathogenicity remains to be fully elucidated. FliC is known to directly elicit an Ipaf-dependent macrophage re-

sponse, leading to macrophage death and inflammation (8, 19). Furthermore, at early stages of infection, FliC expression is restricted to the small intestine and is dramatically repressed by intracellular bacteria (5, 7). We hypothesize that the microenvi-

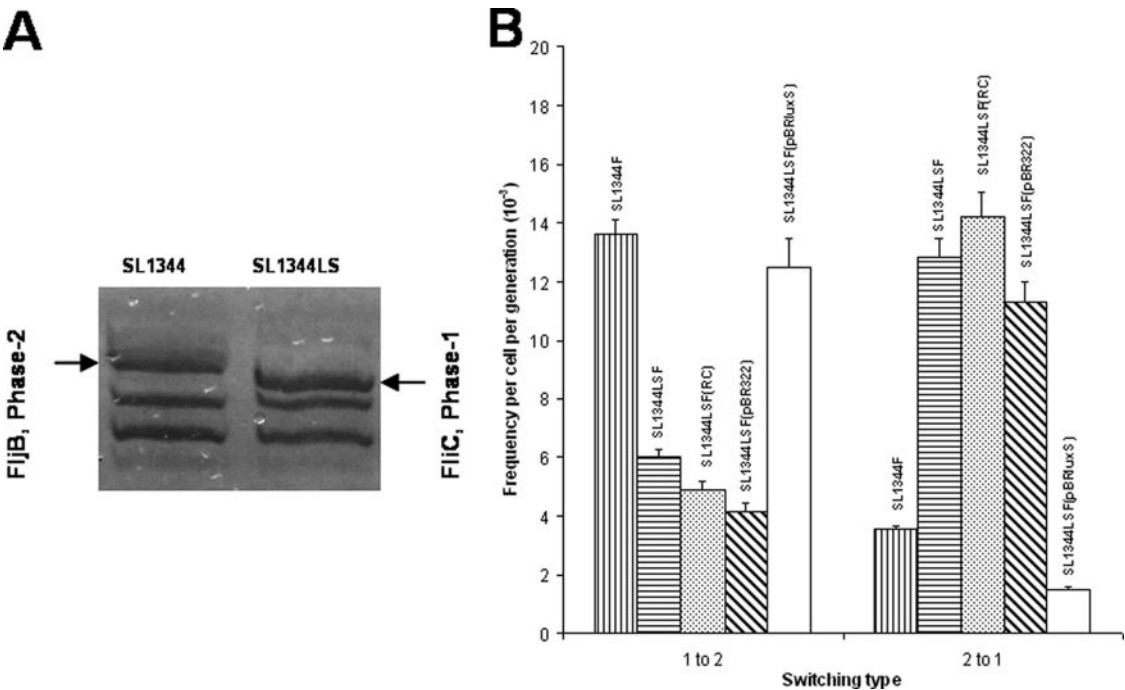


FIG. 1. Flagella phase variation is LuxS dependent in *S. enterica* serovar Typhimurium. (A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of secreted protein from SL1344 and the isogenic *luxS* mutant, SL1344LS, showing the flagellar protein bands identified by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry; (B) rates of flagellar phase switching. Expression of LuxS from pBR*luxS* in SL1344LSF(pBR*luxS*) restores switching frequencies back to parental levels as measured by the TTC plate assay. However, supplementation with reconstituted cell supernatants [SL1344LSF(RC)] containing AI-2 (or other diffusible signaling molecules) fails to restore phase variation back to parental levels in SL1344LSF. Standard errors are shown.

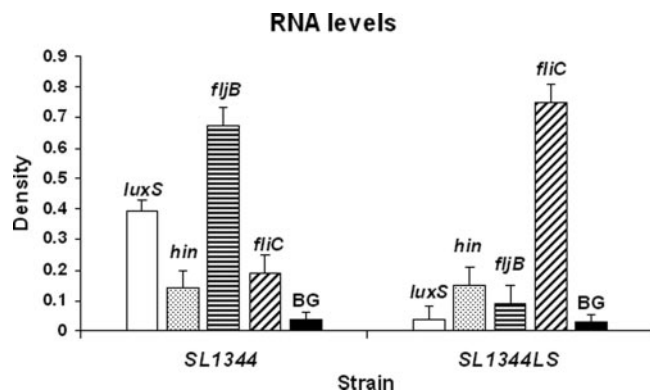


FIG. 2. Transcript levels of the *hin* recombinase are unaffected in SL1344LS. Control transcript (*luxS*) levels are similar to background (BG) levels in SL1344LS, and those of phase 1 (*fliC*) and phase 2 (*fljB*) flagellins appear altered, with phase 1 flagellin predominating in SL1344LS. Standard errors are shown.

ronment within the macrophage may trigger a *luxS*-dependent “stealth” response to enhance expression of the less immunogenic FljB. Phase variation control in *Salmonella* may be delicately balanced by pathways involving *luxS*, creating an advantage to the population under specific environmental conditions.

Quorum sensing allows interbacterial communication to coordinate survival and virulence (12). As well as quorum sensing, *luxS* also has a key metabolic function in the activated methyl cycle (29). Here we demonstrate that in *S. enterica* serovar Typhimurium *luxS* plays a role in flagellar phase variation, highlighting its importance in triggering expression of the less immunogenic FljB and repressing the highly immunogenic FliC. Although *luxS* is an important component of the AI-2-mediated signaling system, in flagellar phase switching we have identified a phenotype independent of diffusible signaling molecules and quorum sensing. Further characterization of the *luxS* signaling pathway in *Salmonella* will allow us to better understand the multiple roles of *luxS* in mediating gene regulation and bacterial fitness.

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